Claims

[1] A laser scan type fluorescence microscope comprising

a laser light source section, an objective lens optical system by which excitation light from the laser light source section is condensed on a sample, a scanning means by which the excitation light from the laser light source section is scanned on a surface of the sample, a pupil projection lens arranged between the scanning means and the objective lens optical system, a detection optical system for detecting fluorescence which is emanated from the sample and has penetrated the objective lens optical system and the pupil projection lens, and the objective lens optical system further comprising an objective lens and an image forming lens for forming an intermediate image of the sample, wherein a backside focal position of the objective lens may become conjugate at a position near the scanning means by the image forming lens and the pupil projection lens, and the following condition is satisfied;

$$0.15 \leqq D / L \leqq 0.5$$

where D is a co-focal length of the objective lens, and L is a distance from the sample surface to the conjugate position of the backside focal position of the objective lens arranged near the scanning means.

[2] The laser scan type fluorescence microscope according to claim 1, comprising an optical transmission means which leads the excitation light from the laser light source section to the scanning means.

[3] The laser scan type fluorescence microscope according to claim 1 or 2, wherein the pupil projection lens consists of two or more lens groups, a concave surface of a lens at the nearest side to the scanning means is directed to the scanning means side, a concave surface of a lens at the nearest side to the intermediate image side is directed to the intermediate image side, and the following condition is satisfied;

$$0.2 \leqq Fe/D3 \leqq 0.5$$

where D3 is a distance from the conjugate position of the pupil of the objective lens located near the scanning means to the intermediate image position of the image forming lens, and Fe is a focal length of the pupil projection lens.

[4] The laser scan type fluorescence microscope according to any one of claims 1 to 3, which consists of two or more lens groups, and comprises at least one cemented lens having a positive lens and a negative lens, and the following conditions are satisfied;

$$0.4 \leqq FTL/D1 \leqq 1$$

$$80 \leqq \nu p$$

where νp is Abbe's number of the positive lens in the cemented lens, FTL is a focal length of the image forming lens, and D1 is a distance from the position of a shoulder of lens to the intermediate image position.

[5] The laser scan type fluorescence microscope according to any one of claims 1 to 4, wherein the image forming lens consists of two lens groups having a front group at the side of an intermediate image and a rear group at the side of an objective lens, and the lens group of the front group of the image forming lens has at least one negative lens, the following conditions are satisfied;

$$0.4 \leqq D2/FTL \leqq 1$$

$$0.7 \leqq FTL1/FTL \leqq 1.5$$

where FTL1 is a focal length of the rear group of the image forming lens, and D2 is an interval between the front group of the image forming lens and the rear group of the image forming lens.

[6] The laser scan type fluorescence microscope according to claim 1, comprising a first multi-mode fiber which leads the excitation light from the laser light source section to the scanning means, a second multi-mode fiber which leads fluorescence from a sample to the detection optical system, a first lens by which entry of the

excitation light to the first multi-mode fiber is carried out, and a second lens by which entry of the fluorescence to the second multi-mode fiber is carried out, and the following conditions are satisfied;

$$2 \leqq \Phi em / \Phi ex \leqq 12$$

$$0.61 \times (\lambda ex / NAex) < \Phi ex$$

$$0.61 \times (\lambda em / NAem) < \Phi em$$

where Φex is a diameter of a core of the first multi-mode fiber, Φem is a diameter of a core of the second multi-mode fiber, NAex is the size of an aperture by which entry to the first multi-mode fiber by the first lens is carried out, λ ex is the excitation wavelength, NAem is the size of an aperture by which entry to the second multi-mode fiber by the second lens is carried out, and λem is the fluorescence wavelength.

[7] The laser scan type fluorescence microscope according to claim 1 or 2, comprising an optical transmission means which leads fluorescence from a sample which transmitted through the pupil projection lens to the detection optical system.

[8] The laser scan type fluorescence microscope according to any one of claims 1 to 7, comprising an optical transmission means by which fluorescence from the sample is lead to the detection optical system, while excitation light from the laser light source section is led to the scanning means.

[9] The laser scan type fluorescence microscope according to any one of claims 1 to 7, comprising a first optical transmission means which leads excitation light from the laser light source section to the scanning means, and a second optical transmission means which leads fluorescence from the sample mentioned above to the detection optical system.

[10] The laser scan type fluorescence microscope according to any one of claims 1 to 9,

wherein the objective lens is a submerged type objective lens.

[11] The laser scan type fluorescence microscope according to any one of claims 1 to 7, wherein the laser light source consists of a semiconductor laser.

[12] The laser scan type fluorescence microscope according to any one of claims 1 to 7, wherein the detector is constituted on the main body portion of a microscope.

[13] The laser scan type fluorescence microscope according to claim 1, comprising a first multi-mode fiber which leads the excitation light from the laser light source section to the scanning means, a second multi-mode fiber which leads fluorescence from a sample to the detection optical system, a first lens by which entry of the excitation light to the first multi-mode fiber is carried out and a second lens in which entry of the fluorescence of the second multi-mode fiber is carried out, wherein all of the following conditions are satisfied;

$$4 \leqq \Phi em / \Phi ex \leqq 10$$

$$0.61 \times (\lambda ex / NAex) < \Phi ex$$

$$0.61 \times (\lambda em / NAem) < \Phi em$$

where Φex is a diameter of a core of the first multi-mode fiber, Φem is a diameter of a core of the second multi-mode fiber, NAex is the size of an aperture by which entry to the first multi-mode fiber by the first lens is carried out, λex is the excitation wavelength, NAem is the size of an aperture by which entry to the second multi-mode fiber by the second lens is carried out, and λem is the fluorescence wavelength.

[14] The laser scan type fluorescence microscope comprising a laser light source section, an objective lens optical system which condenses excitation light from the laser light source section on a sample, a scanning means which scans the excitation

light from the laser light source section on the sample surface, a pupil projection lens arranged between the scanning means and the objective lens optical system, and a detection optical system which detects fluorescence emanated from the sample and transmitted through the objective lens optical system and the pupil projection lens, wherein it further comprises a first multi-mode fiber which leads the excitation light from the laser light source section to the scanning means, a second multi-mode fiber which leads fluorescence from a sample to the detection optical system, a first lens by which entry of the excitation light to the first multi-mode fiber is carried out, and a second lens in which entry of the fluorescence of the second multi-mode fiber is carried out, wherein all of the following conditions are satisfied;

$$2 \leqq \Phi em / \Phi ex \leqq 12$$

$$0.61 \times (\lambda ex / NAex) < \Phi ex$$

$$0.61 \times (\lambda em / NAem) < \Phi em$$

where Φex is a diameter of a core of the first multi-mode fiber, Φem is a diameter of a core of the second multi-mode fiber, NAex is the size of an aperture by which entry to the first multi-mode fiber by the first lens is carried out, λex is the excitation wavelength, NAem is the size of an aperture by which entry to the second multi-mode fiber by the second lens is carried out, and λem is the fluorescence wavelength.